09/889993

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860.00	JC18 Rec'd PCT/PTO 23 JUL 2001	
International preliminary examination fee paid to USPTO (37 CFR 1.482) $0.00		
No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $0.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $0.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	16-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
Intellectual Property/Technology Law
P. O. Box 14329
Research Triangle Park, NC 27709

Marianne Fuierer
MARIANNE FUIERER
Registration No. 39,983

09/889993

17.. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860.00	JC18 Rec'd PCT/PTO 23 JUL 2001	
International preliminary examination fee paid to USPTO (37 CFR 1.482) $0.00		
No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $0.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $0.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	16-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
Intellectual Property/Technology Law
P. O. Box 14329
Research Triangle Park, NC 27709

MARIANNE FUIERER
Registration No. 39,983

4121-127
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**SCHULZE-GARG, et al.**
Application No.:	New U.S. National Stage Application of PCT International Application No. PCT/DE00/00232
International Filing Date:	26 January 2000
Priority Date Claimed:	28 January 1999 (German Appl. No. 199 03 371.4)
U.S. National Phase Filing Date:	Date of mailing identified below
Title:	**MAMMAL, METHOD FOR PRODUCING SAME AND ITS USE**

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Box Patent Application, Washington, DC 20231, and Express Mailed under the provisions of 37 CFR 1.10.

Lee Ann Brown
Name of Person Mailing This Document

Lee Ann Brown
Signature

July 23, 2001
Date

EL831358554US
Express Mail Label Number

PRELIMINARY AMENDMENT

Commissioner for Patents
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

In the Specification

On the top of page 3, please replace the first paragraph with the following amended paragraph:

The expression "oncogene" comprises any gene or portions thereof which may have a cell-transforming property. Examples of such genes are erb A, erb B, fos, myc, E6, E7 and the early region of SV40, i.e. the gene for SV40 T-Ag, as well as mutated p53. The oncogene may also comprise a nucleotide sequence (SEQ ID NO: 1) coding for a strong, i.e. immunodominant, T-cell epitope, e.g. the MHCI-restricted epitope n118 of the LCM virus nucleoprotein (SEQ ID NO: 2).

On the bottom of page 3, please replace the last paragraph with the following amended paragraph.

Preferred mammals of the present invention are mice which contain the gene for the SV40 T-Ag under the control of the WAP promoter. The SV-40 T-Ag gene may also contain a nucleotide sequence (SEQ ID NO: 1) coding for a strong, i.e. immunodominant, T-cell epitope, e.g. epitope n118 of the LCM virus nucleoprotein (SEQ ID NO: 2). Such mice are referred to as WAP-T or WAP-T-NP (cf. figure 1). The mice WAP-T-1, WAP-T-2, WAP-T-10, WAP-T-NP6, WAP-T-NP8 and WAP-T-NP10 are preferred. These mice are distinguished as follows:

In the Claims

Please amend claims 1-16 to read as follows:

1. A mammal with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that can be activated by lactotropic hormones and comprises a nucleotide sequence coding for a strong T-cell epitope, the nucleotide sequence being SEQ ID NO: 1.

2. The mammal according to claim 1, wherein the oncogene is controlled by the WAP promoter.

3. The mammal according to claim 1, wherein the oncogene is a gene coding for SV40 T-Ag.

4. The mammal according to claim 1, wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein having the amino acid sequence of SEQ ID NO: 2.

5. The mammal according to claim 3, wherein the mammal is selected from the group consisting of WAO-T-NP6, WAP-T-NP8 and WAP-T-NP10.

6. The mammal according to claim 1 with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that can be activated by lactotropic hormones and is selected from the group consisting of WAP-T-1, WAP-T-2 and WAP-T-10.

7. The mammal according to claim 1, wherein DCIS develops into an invasive ductal mammary carcinoma.

8. The mammal according to claim 1, wherein the lactotropic hormones are estrogen, prolactin, insulin, and hydrocortisone.

9. A method of providing a mammal that contains an oncogene that can be activated by lactotropic hormones, comprising the steps of:
   (a) introducing a DNA coding for an oncogene into inseminated oocytes of a mammal, the DNA code being SEQ ID NO: 1 and being controlled by a promoter specific to lactotropic horomones,
   (b) implanting the oocytes from (a) into pseudopregnant mammals, and
   (c) selecting the progeny obtained in (b) for the formation of DCIS.

10. The method according to claim 9, wherein the promoter is the WAP promoter.

11. The method according to claim 9, wherein the oncogene is a gene coding for SV40 T-Ag.

12. The method according to claim 9, wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein having the amino acid sequence of SEQ ID NO: 2.

13. The method according to claim 12, wherein the lactotropic hormones comprise estrogen, prolactin, insulin and hydrocortisone.

14. The method according to claim 9, wherein DCIS develops

into invasive ductal mammary carcinoma.

15. Use of the mammal according to claim 1 for studying DCIS, its progression towards an invasive ductal carcinoma and the latter.

16. Use of the mammal according to claim 1 for the research and development of diagnostic markers and therapeutic agents for a DCIS or an invasive ductal carcinoma.

REMARKS

A marked-up version of amended paragraph in the specification and amended claims 1-16 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-16.

Respectfully submitted,

Marianne Fuierer
Registration No. 39,983
Attorney for Applicants

INTELLECTUAL PROPERTY/ TECHNOLOGY LAW
P. O. Box 14329
Research Triangle Park, NC 27709
Phone: (919) 419-9350
Fax: (919) 419-9354
Attorney File: 4121-127

APPENDIX A

In the Specification

On the top of page 3, please replace the first paragraph with the following amended paragraph:

The expression "oncogene" comprises any gene or portions thereof which may have a cell-transforming property. Examples of such genes are erb A, erb B, fos, myc, E6, E7 and the early region of SV40, i.e. the gene for SV40 T-Ag, as well as mutated p53. The oncogene may also comprise a nucleotide sequence[s] (SEQ ID NO: 1) coding for a strong, i.e. immunodominant, T-cell epitope, e.g. the MHCI-restricted epitope n118 of the LCM virus nucleoprotein (SEQ ID NO: 2).

On the bottom of page 3, please replace the last paragraph with the following amended paragraph.

Preferred mammals of the present invention are mice which contain the gene for the SV40 T-Ag under the control of the WAP promoter. The SV-40 T-Ag gene may also contain a nucleotide sequence[s] (SEQ ID NO: 1) coding for a strong, i.e. immunodominant, T-cell epitope, e.g. epitope n118 of the LCM virus nucleoprotein (SEQ ID NO: 2). Such mice are referred to as WAP-T or WAP-T-NP (cf. figure 1). The mice WAP-T-1, WAP-T-2, WAP-T-10, WAP-T-NP6, WAP-T-NP8 and WAP-T-NP10 are preferred. These mice are distinguished as follows:

In the Claims

1. A mammal with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that

can be activated by lactotropic hormones and comprises a nucleotide sequence coding for a strong T-cell epitope, the nucleotide sequence being SEQ ID NO: 1.

2. The mammal according to claim 1, wherein the oncogene is controlled by the WAP promoter.

3. The mammal according to claim 1 [or 2], wherein the oncogene is a gene coding for SV40 T-Ag.

4. The mammal according to claim 1 [any of claims 1 to 3], wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein having the amino acid sequence of SEQ ID NO: 2.

5. The mammal according to claim 3 [any of claims 1 to 4], wherein the mammal is selected from the group consisting of WAO-T-NP6, WAP-T-NP8 and WAP-T-NP10. [those of figures 7, 8 and 9.]

6. The mammal according to claim 1 with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that can be activated by lactotropic hormones and is selected from the group consisting of WAP-T-1, WAP-T-2 and WAP-T-10. [those of figures 4, 5 and 6.]

7. The mammal according to claim 1 [any of claims 1 to 6], wherein DCIS develops into an invasive ductal mammary carcinoma.

8. The mammal according to claim 1 [any of claims 1 to 7], wherein the lactotropic hormones are estrogen, prolactin, insulin, and hydrocortisone.

9. A method of providing a mammal that contains an oncogene that can be activated by lactotropic hormones [according to any of claims 1 to 5], comprising the steps of:

(a) introducing a DNA coding for an oncogene into inseminated oocytes of a mammal, the DNA code being SEQ ID NO: 1 and being controlled by a promoter specific to lactotropic horomones,

(b) implanting the oocytes from (a) into pseudopregnant mammals, and

(c) selecting the progeny obtained in (b) for the formation of DCIS.

10. The method according to claim 9, wherein the promoter is the WAP promoter.

11. The method according to claim 9 [or 10], wherein the oncogene is a gene coding for SV40 T-Ag.

12. The method according to claim 9 [any of claims 9 to 11], wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein having the amino acid sequence of SEQ ID NO: 2.

13. The method according to claim 12 [any of claims 9 to 12], wherein the lactotropic hormones comprise estrogen, prolactin, insulin and hydrocortisone.

14. The method according to claim 9 [any of claims 9 to 13], wherein DCIS develops into invasive ductal mammary carcinoma.

15. Use of the mammal according to claim 1 [any of claims 1 to 8] for studying DCIS, its progression towards an invasive ductal carcinoma and the latter.

16. Use of the mammal according to claim 1 [any of claims 1 to 8] for the research and development of diagnostic markers and therapeutic agents for a DCIS or an invasive ductal carcinoma.

23448
PATENT TRADEMARK OFFICE

4121-127
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: SCHULZE-GARG, et al.

Application No.: 09/889,993

International Application No.: PCT/DE00/00232

Priority Dates Claimed: 26 January 2000 and 28 January 1999 (German Appl. No. 199 03 371.4)

Title: MAMMAL, METHOD FOR PRODUCING SAME AND ITS USE

FIRST CLASS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.8.

Signature of Person Signing

November 14, 2001
Date of Mailing

SUPPLEMENTAL PRELIMINARY AMENDMENT IN U.S. PATENT APPLICATION 09/889,993

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified national phase patent application, please amend the application, as follows:

In the Specification

Please insert on page 1 between the title of the application and the first paragraph the following new paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under the provisions of 35 USC §371 and claims the priority of International Patent Application No. PCT/DE00/00232 filed January 26, 2000, which in turn claims priority of German Patent Application No. 199 03 371.4 filed January 28, 1999.

REMARKS

This claim to priority is being filed within four (4) months of the above-identified application meeting all requirements under 35 U.S.C. §371(b).

Respectfully submitted,

Marianne Fuierer

Marianne Fuierer
Registration No. 39,983
Attorney for Applicants

**INTELLECTUAL PROPERTY/
TECHNOLOGY LAW
P. O. Box 14329
Research Triangle Park, NC 27709
Phone: (919) 419-9350
Fax: (919) 419-9354
Attorney File: 4121-127**

M 4452

Mammal, Method for Producing Same and Its Use

The present invention relates to a mammal in which a ductal carcinoma *in situ* (DCIS) of the female mammary gland can be induced. The invention also relates to a method of producing such a mammal and its use for studying DCIS and/or its progression towards an invasive ductal carcinoma of the breast, and for the development of diagnostic and/or therapeutic means for this purpose.

The mammary carcinoma which occurs with a frequency of about 10 % among the female population is one of the pressing health problems of our time. About 80 % of the mammary carcinomas are invasive ductal mammary carcinomas. Mammary tumors belonging to the type of ductal carcinoma *in situ* (DCIS) are frequently diagnosed in a mammography. DCIS is characterized by a non-invasive neoplastic proliferation, i.e. a proliferation not yet breaking through the basal membrane, of epithelial cells into the lumen of the ductulo-lobulary unit of the mammary system. A DCIS may develop into an invasive ductal mammary carcinoma. The molecular causes of this are, however, not known. Likewise, a prediction as to whether and when such a development occurs is not possible either. As a result, radical mastectomy, i.e. complete removal of the female breast and local lymph nodes, is usually carried out when DCIS of the female breast is diagnosed. Estimates, however, show that about 60 % of the radical mastectomies represent an excessive treatment.

It is thus the object of the present invention to provide a product by which the molecular causes of DCIS, in particular its progression towards an invasive ductal mammary carcinoma, can be studied and optionally possibilities for a reliable diagnosis and/or appropriate therapy can be shown.

According to the invention this is achieved by the subject

matters defined in the claims.

The present invention is based on Applicant's insights that DCIS of the female mammary gland can be induced in mammals, e.g. mice, containing an oncogene, e.g. the early region of SV40, i.e. the gene for the SV40 T-Ag, which can be activated by lactotropic hormones such as estrogen, prolactin, insulin and hydrocortisone. He also found that DCIS may develop into an invasive ductal mammary carcinoma.

According to the invention, Applicant's insights are used to provide a mammal having an inducible DCIS of the female mammary gland, which contains an oncogene that can be activated by lactotropic hormones. DCIS may preferably develop into an invasive ductal mammary carcinoma.

The expression "DCIS of the female mammary gland" refers to a non-invasive neoplastic proliferation, i.e. a proliferation which does not yet break through the basal membrane, of epithelial cells into the lumen of the ductulo-lobulary unit of the mammary gland system. In particular, DCIS distinguishes itself by histological features, such as hyperchromatic, pleomorphous, large-scale structured or strikingly large nuclei. It may show a shifted nucleus-plasma relation or numerous mitotic patterns. DCIS may also be characterized in that the proliferation of the epithelial cells into the lumen of the duct manifests itself as a multi-layered or sievelike lining or as an intraluminal branching or by means of micropapillae. Moreover, DCIS may show as necroses, apoptosis patterns, psammoma bodies, i.e. onion skin-like crystallization products having calcifications, in the lumen of the duct and loss of the myoepithelial layer underneath the basal membrane.

The expression "lactotropic hormones" refers to hormones which are released by mammals, e.g. during pregnancy and/or lactation, and have a lactotropic effect. Examples of such hormones are estrogen, prolactin, insulin and hydrocortisone.

The expression "oncogene" comprises any gene or portions thereof which may have a cell-transforming property. Examples of such genes are erb A, erb B, fos, myc, E6, E7 and the early region of SV40, i.e. the gene for SV40 T-Ag, as well as mutated p53. The oncogene may also comprise sequences coding for a strong, i.e. immunodominant, T-cell epitope, e.g. the MHCI-restricted epitope n118 of the LCM virus nucleoprotein.

The expression "oncogene that can be activated by lactotropic hormones" refers to the fact that the above oncogene can be activated by lactotropic hormones. This may be achieved in the most differing ways. It may be favorable for the oncogene to be controlled by a promoter which is specific to one or more lactotropic hormones. Such a promoter is e.g. the "whey acidic protein" (WAP) promoter. Its specificity comprises the lactotropic hormones estrogen, prolactin, insulin and hydrocortisone. Reference is made to the below description regarding the production of a mammal according to the invention.

The expression "mammal" comprises any animals, with the exception of humans, which release lactotropic hormones, e.g. during pregnancy and/or lactation, and which may contain an oncogene that can be activated by lactotropic hormones. Examples of such mammals are mice, rats, rabbits, horses, bovine animals, sheep, goats, monkeys, pigs, dogs and cats, mice being mentioned above all.

Preferred mammals of the present invention are mice which contain the gene for the SV40 T-Ag under the control of the WAP promoter. The SV-40 T-Ag gene may also contain sequences coding for a strong, i.e. immunodominant, T-cell epitope, e.g. epitope n118 of the LCM virus nucleoprotein. Such mice are referred to as WAP-T or WAP-T-NP (cf. figure 1). The mice WAP-T-1, WAP-T-2, WAP-T-10, WAP-T-NP6, WAP-T-NP8 and WAP-T-NP10 are preferred. These mice are distinguished as follows:

WAP-T-1

These mice usually develop multifocal invasive ductal mammary

carcinoms after an average of 7 months following the induction with lactotropic hormones. No preferential tumor formation can be observed in one of the mammae. Invasive carcinomas are, as a rule, differentiated tubularly to papillarily and have partially solid anaplastic portions which may also show a desmoplastic reaction. Pulmonary metastases occur occasionally. However, the majority of animals does not form metastases, and the primary tumors only grow slowly. Macroscopically non-affected mammae show multifocal DCIS. Due to the isomorphous manifestation of the nuclei most DCIS may be classified as "low grade" in analogy to the classification of human DCIS according to the NUYS index. Comedonecroses and psammoma bodies are observed from time to time. However, there is a formation of intraluminal branches ("roman arches" formation) (cf. figure 4).

WAP-T-2

These animals have a light-brown coat. Furthermore, they develop invasive ductal mammary carcinomas about 6 months after the induction with lactotropic hormones. Differentiated mammary carcinomas are predominantly tubular to lobular. Anaplastic mammary carcinomas appear with some tumor giant cells. Micrometastases in lymph nodes are observed. In rare cases, fibrosarcomas may form, starting from mammary or uterus. The multifocally occurring DCIS show micropapillary and cribriform growth patterns. Forms which have monolayered lining and comdeonecrosis or psammoma bodies are also observed. Since the cell nuclei are usually isomorphous, these DCIS cannot be assessed as "high grade" (cf. above) (cf. figure 5).

WAP-T-10

About 8 months after the induction with lactotropic hormones, these mice develop palpable ductal mammary carcinomas which may form metastases. Both solid carcinomas and carcinomas with little differentiation which have numerous mitoses as well as tubular to papillary forms may be found. The investigated metastases are differentiated papillarily. The multifocally occurring DCIS have comedonecroses and due to

the morphology of nuclei (pleomorphism, hyperchromasia, and others) correspond to a "high grade" DCIS (see above; and figure 6).

WAP-T-NP6

These mice develop palpable invasive carcinomas about 11 months after multiple induction with lactotropic hormones. In rare cases, hepatocellular adenomas and adenomas of the salivary gland occur as well. The mammary carcinomas are differentiated predominantly tubularly or papillarily, in some cases they are differentiated only moderately with extensive necroses and they are partially also solid. Micrometastases occur in mammary lymph nodes. The cell nuclei of the multifocally occurring DCIS are usually inconspicuously "low grade" (see above) and comedonecroses are present (cf. figure 7).

WAP-T-NP8

These mice develop invasive ductal carcinomas about 5 months following induction with lactotropic hormones. Both tubulo-papillarily differentiated and poorly differentiated solid tumors are found. Animals having poorly differentiated tumors show pulmonary metastases. The lumens of localized small invasive carcinomas and of DCIS are in some cases infiltrated by granulocytes. Non-epithelial tumors of mammary origin, e.g. fibrosarcomas and osteosarcomas, and infiltrating histiocytic sarcomas also occur occasionally. The multifocal DCIS apparent 15 to 20 weeks following induction with lactotropic hormones correspond to a "high grade" form due to the morphology of cell nuclei (see above; and figure 8).

WAP-T-NP10

These mice develop an invasive ductal mammary carcinoma about 11 months following several inductions with lactotropic hormones. These carcinomas are frequently differentiated tubularly to papillarily and have solid and necrotic but only moderately differentiated portions. The DCIS appear with isomorphous nuclei (not "high grade"; see above). DCIS having micropapillary growth and forms with total loss of the

myoepithelial layer and psammoma body formation occur (cf. figure 9).

Another subject matter of the present invention relates to cells which are obtained from the mammal according to the invention. These cells may be present in any form, e.g. in a primary or long-term culture.

A mammal according to the invention may be provided by common methods. A method may be favorable which comprises the steps of:

(a) introducing a DNA coding for an oncogene into inseminated oocytes of a mammal, the DNA being controlled by a promoter specific to lactotropic hormones,
(b) implanting the oocytes from (a) into pseudo-pregnant mammals, and
(c) selecting the progeny obtained in (b) for the formation of a DCIS.

As to the expressions "oncogene", "lactotropic hormones", "mammals" and "DCIS" reference is made to the above explanations.

Furthermore, the expression "pseudopregnant mammals" refers to mammals which were paired with non-potent, i.e. sterile or vasectomized, male mammals, and have a vaginal plug. Reference is made to the below example.

The expression "inseminated oocytes" refers to oocytes of pregnant mammals which have been emptied. Reference is made to the below example.

The expression "DNA coding for an oncogene and controlled by a promoter specific to lactotropic hormones" relates to a DNA present in any form and having these properties. The DNA may be present as such or in combination with another DNA, e.g. a vector. It may also be circular or linear. Furthermore, it

may contain sequences supporting a recombination with the DNA of the mammal. In addition, it may contain sequences which code for a T-cell epitope, e.g. the MHC I restricted epitope n118 of the LCM virus nucleoprotein. Such a DNA was deposited with DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen)* [German-type collection of microorganisms and cell cultures] as pWAP-T-NP under DSM 12608 on December 22, 1998.

The person skilled in the art also knows conditions and materials to carry out steps (a) - (c). As to the selection in (c) he will use e.g. methods by means of which the above-mentioned histologic features can be detected.

A mammal is provided by the present invention, in which DCIS can be induced. Furthermore, an invasive ductal mammary carcinoma can develop from DCIS. The molecular causes of DCIS or its progression towards an invasive ductal mammary carcinoma may thus be studied. In particular, it is of advantage that mammals can be provided which have differently long latent periods until DCIS or an invasive ductal carcinoma has been developed, so that by means of a comparative study a correlation can be made between DCIS type (including the identified molecular markers) and the risk of DCIS. It is also of advantage that the role of the immune system in the development of DCIS or its progression towards an invasive ductal mammary carcinoma can be studied, which is supported by the presence of a strong T-cell epitope in the oncogene product. Besides, the present invention provides a basis for the development of diagnostic markers by which individual development levels of DCIS or the invasive ductal carcinoma can be detected and thus predictions can be made regarding the development of DCIS or its progression. The present invention also provides the possibility of developing therapeutic agents against the above diseases.

Brief description of the drawings:

Figure 1 shows a DNA used for the production of a mouse

according to the invention and coding for the SV40 T-Ag or the SV40 T-Ag containing the n118 epitope of the LCM virus (SV40 T-Ag-NP). The DNA is used in a linear form and injected into inseminated oocytes.

Figure 2 shows the expression of SV40 T-Ag or SV40 T-Ag-NP in the mammary glands of mice according to the invention.

(a) Nuclear expression of SV40 T-Ag in the epithelial cells of morphologically inconspicuous ductuli 12 months following the induction of the WAP-T-1 mouse according to the invention.

(b) Nuclear expression of T-Ag in the tumor cells of DCIS 12 months following the induction of the WAP-T-NP6 mouse according to the invention.

Figure 3 shows examples of frequent tumor phenotypes of mice according to the invention. Sections embedded in paraffin, H&E staining;

(a) Invasive ductal mammary carcinoma differentiated tubularly to papillarily and with desmoplastic reaction of the WAP-T-10 mouse.

(b) Anaplastic invasive ductal mammary carcinoma with extensive necroses of the WAP-T-NP8 mouse.

Figure 4 shows induced DCIS with relatively inconspicuous nuclei in the DCIS mouse WAP-T-1, in part with intraluminal branching, psammoma bodies and comedonecrosis.

Figure 5 shows in the DCIS mouse WAP-T-2 induced DCIS with micropapillary growth pattern, desmoplastic reaction and inflammatory infiltrates, in addition a DCIS with monolayered to multi-layered lining as

well as psammoma bodies and comedonecrosis.

Figure 6 shows several induced DCIS with hyperchromatic pleomorphous nuclei in the DCIS mouse WAP-T-10. Inflammatory infiltrates can be detected in the vicinity.

Figure 7 shows an induced DCIS with usually inconspicuous nuclei and occasional comdeonecroses in the DCIS mouse WAP-T-NP6. Mono-layered and locally multilayered linings of the ductal lumens are detectable.

Figure 8 shows several induced DCIS with pleomorphous, partially strikingly large nuclei and psammoma bodies in the DCIS mouse WAP-T-NP8.

Figure 9 shows an induced DCIS with a growth pattern forming relatively isomorphous nuclei and micropapillary and partially intraluminal branchings in the DCIS mouse WAP-T-NP10.

The invention is explained by the example.

Example: Production of a mammal according to the invention

About 20 female CB6F1 mice at the age of 4 to 5 weeks are superovulated by intraperitoneal injection of 5 U PMS (pregnant mare's serum) on day 1 and another intraperitoneal injection of 5 U hCG (human chorionic gonadotropin) on day 3 and are paired with male CB6F1 animals in the evening of that very day. In the morning of the 4^{th} day, the animals are investigated for the presence of a vaginal plug, positive animals are killed by cervical dislocation and the oviducts are removed. The oocytes are removed from the oviducts and placed into M2 medium, the cumulus cells are separated by short incubation using hyaluronidase, the oocytes are washed thoroughly and stored in an incubator (5 % CO_2, 85 % humidity, 37°C) in M16 medium covered with paraffin oil until they are

microinjected.

The DNA of figure 1 is usually injected into the male pronucleus of inseminated oocytes on the 4th day. Injected oocytes are then incubated in the incubator up to the retransfer taking place the following day. For providing pseudopregnant foster mice, about 25 female B6CBAF1 mice at the age of 8 to 12 weeks are paired with vasectomized male mice in the evening before the microinjection. In the morning of the 5th day, the animals with vaginal plug are selected for retransfer of the injected oocytes. The microinjected oocytes cultured overnight and proliferated to the two-cell stage are reimplanted on the 5th day. In this connection, 10-15 embryos are rinsed into the infundibulum of an oviduct of a narcotized foster mouse. 19-20 days after the retransfer the implanted embryos are born. The mice shown in figures 4-9 are obtained.

M 4452

Amended Claims

1. A mammal with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that can be activated by lactotropic hormones and comprises a sequence coding for a strong T-cell epitope.

2. The mammal according to claim 1, wherein the oncogene is controlled by the WAP promoter.

3. The mammal according to claim 1 or 2, wherein the oncogene is a gene coding for SV40 T-Ag.

4. The mammal according to any of claims 1 to 3, wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein.

5. The mammal according to any of claims 1 to 4, wherein the mammal is selected from those of figures 7, 8 and 9.

6. The mammal with inducible ductal carcinoma *in situ* (DCIS), wherein the mammal contains an oncogene that can be activated by lactotropic hormones and is selected from those of figures 4, 5 and 6.

7. The mammal according to any of claims 1 to 6, wherein DCIS develops into an invasive ductal mammary carcinoma.

8. The mammal according to any of claims 1 to 7, wherein the lactotropic hormones are estrogen, prolactin, insulin, and hydrocortisone.

9. A method of providing a mammal according to any of claims 1 to 5, comprising the steps of:

(a) introducing a DNA coding for an oncogene into

inseminated oocytes of a mammal, the DNA being controlled by a promoter specific to lactotropic horomones,

(b) implanting the oocytes from (a) into pseudopregnant mammals, and

(c) selecting the progeny obtained in (b) for the formation of DCIS.

10. The method according to claim 9, wherein the promoter is the WAP promoter.

11. The method according to claim 9 or 10, wherein the oncogene is a gene coding for SV40 T-Ag.

12. The method according to any of claims 9 to 11, wherein the sequence codes for the n118 epitope of the LCM virus nucleoprotein.

13. The method according to any of claims 9 to 12, wherein the lactotropic hormones comprise estrogen, prolactin, insulin and hydrocortisone.

14. The method according to any of claims 9 to 13, wherein DCIS develops into invasive ductal mammary carcinoma.

15. Use of the mammal according to any of claims 1 to 8 for studying DCIS, its progression towards an invasive ductal carcinoma and the latter.

16. Use of the mammal according to any of claims 1 to 8 for the research and development of diagnostic markers and therapeutic agents for a DCIS or an invasive ductal carcinoma.

Abstract of the Disclosure

The present invention relates to a mammal with inducible ductal carcinoma *in situ* (DCIS), the mammal containing an oncogene which can be activated by lactotropic hormones. The invention also relates to a method of producing such a mammal and its use for studying a DCIS or the progression thereof towards invasive ductal carcinoma of the breast as well as for developing diagnostic and therapeutic means for this purpose.

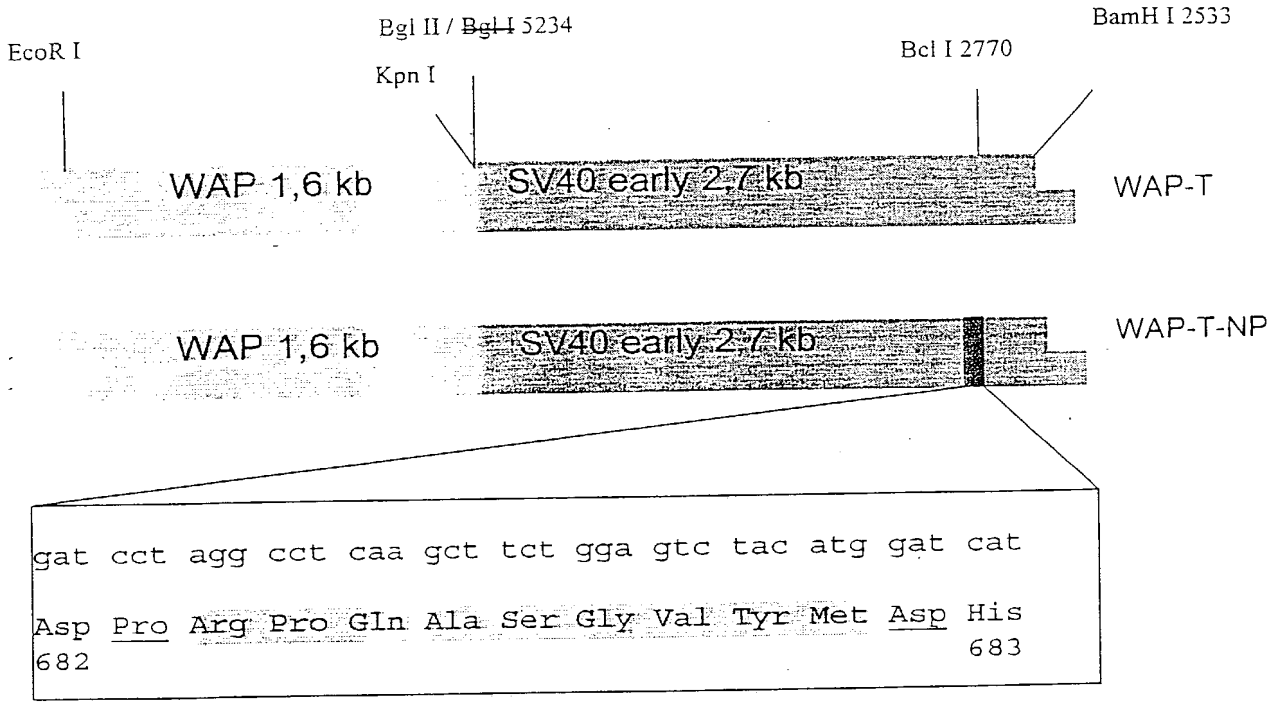
EcoR I
Bgl II / Bgl I 5234
Kpn I
Bcl I 2770
BamH I 2533
WAP 1,6 kb
SV40 early 2,7 kb
WAP-T
WAP 1,6 kb
SV40 early 2,7 kb
WAP-T-NP
gat cct agg cct caa gct tct gga gtc tac atg gat cat
Asp Pro Arg Pro Gln Ala Ser Gly Val Tyr Met Asp His
682
683

Fig. 1

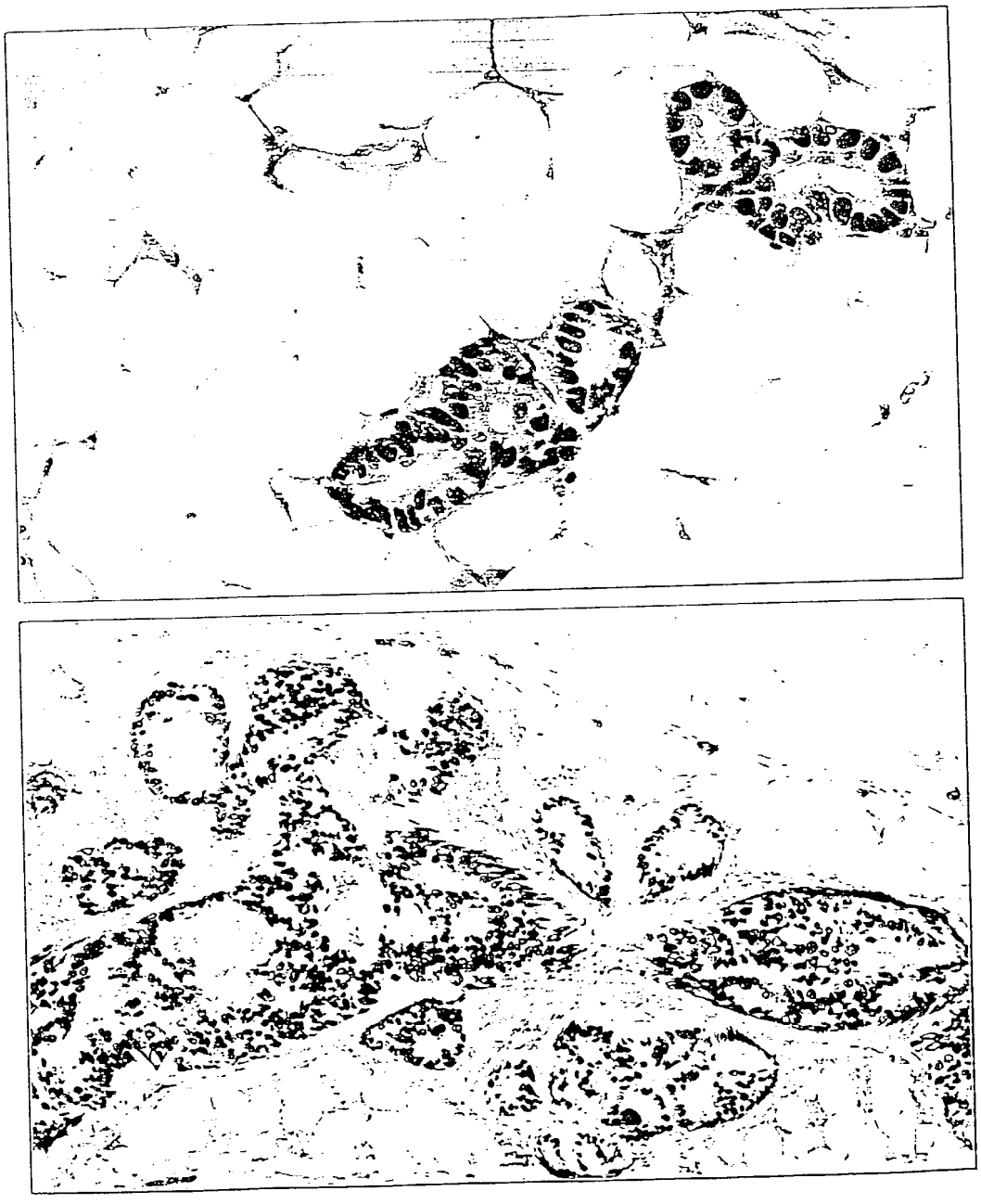

Fig. 2

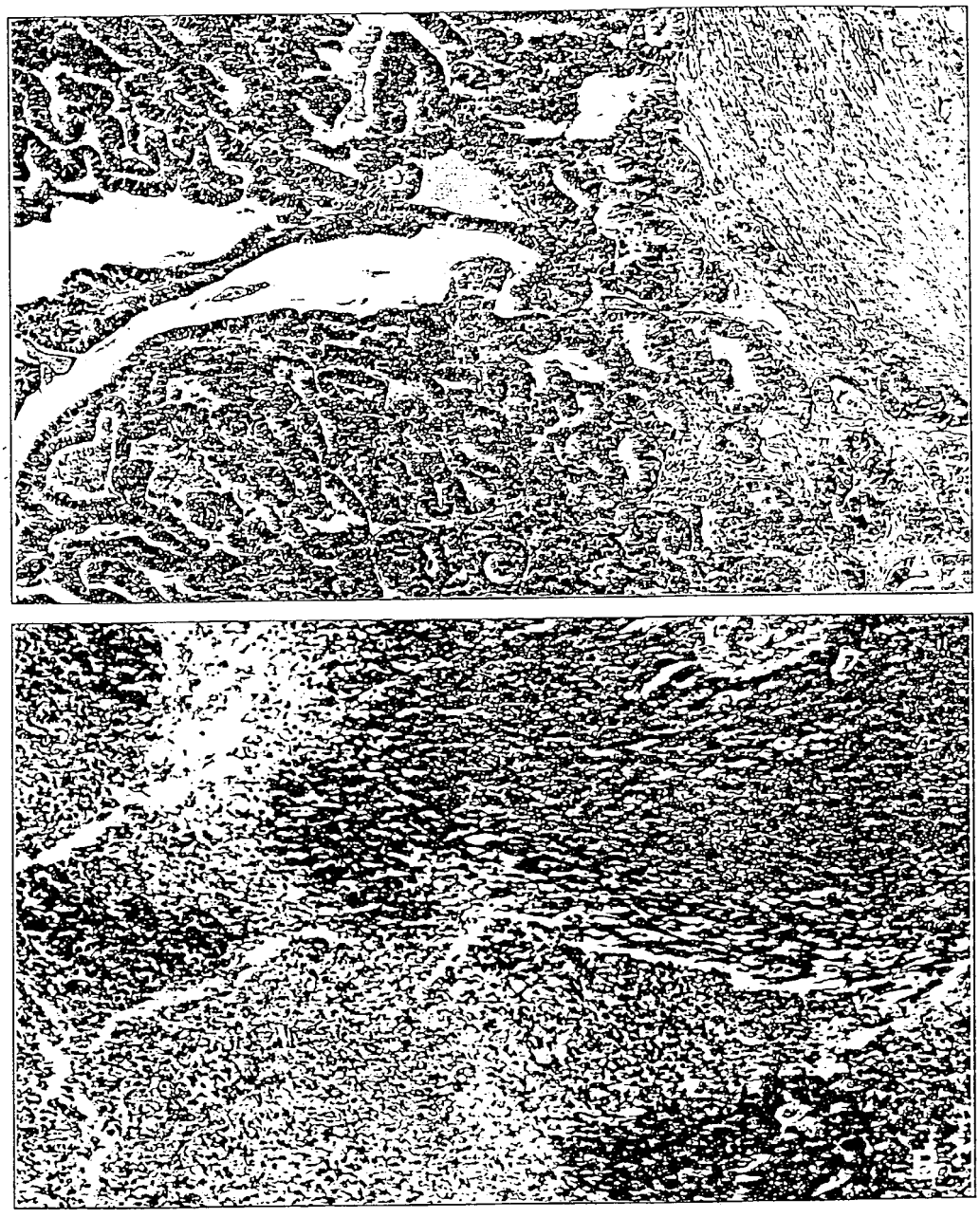

Fig. 3

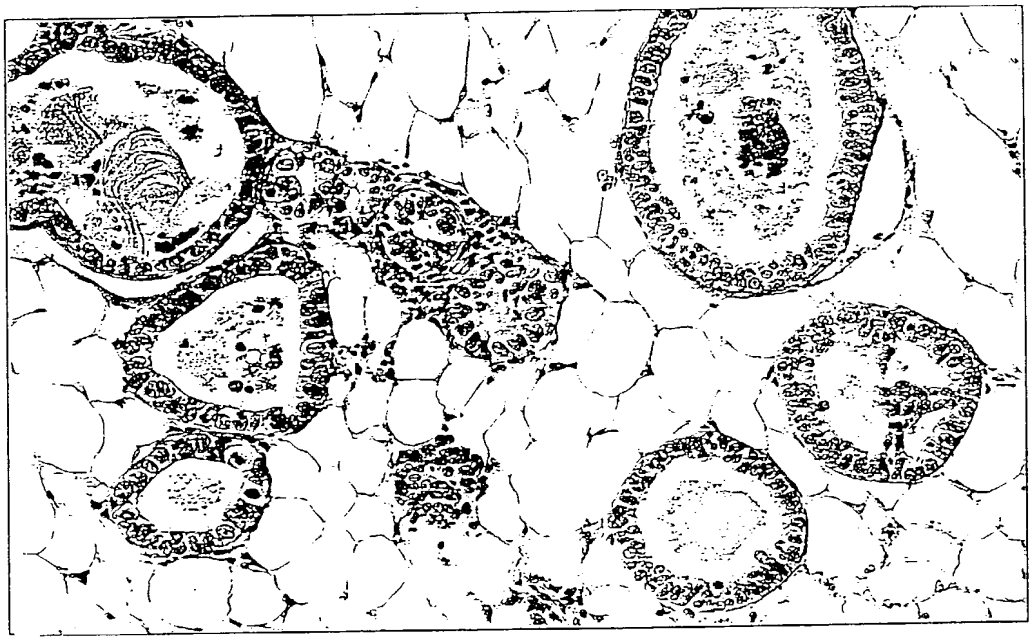

Fig. 4

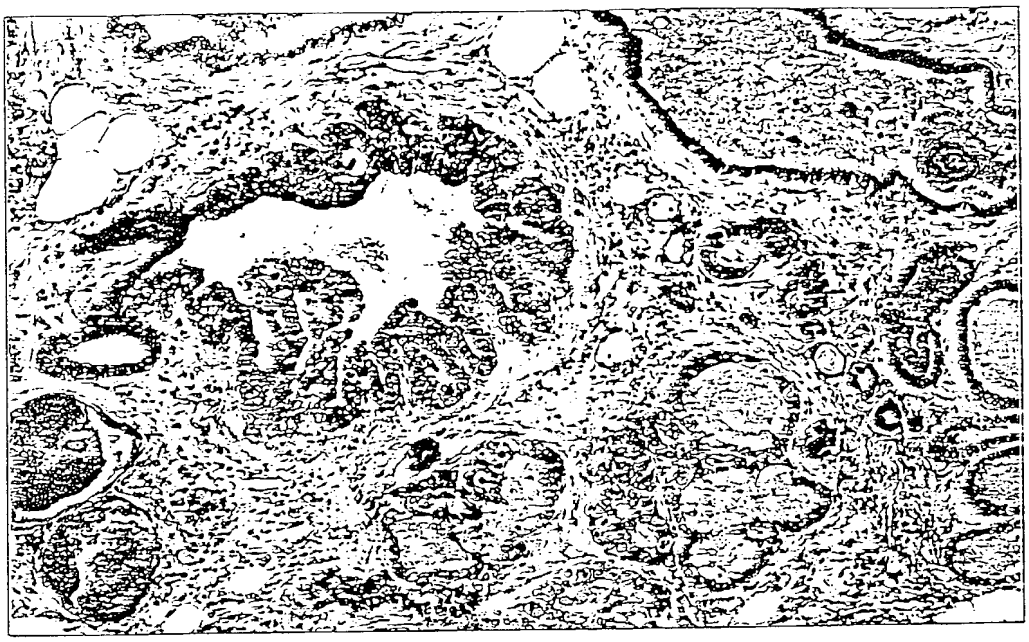

Fig. 5

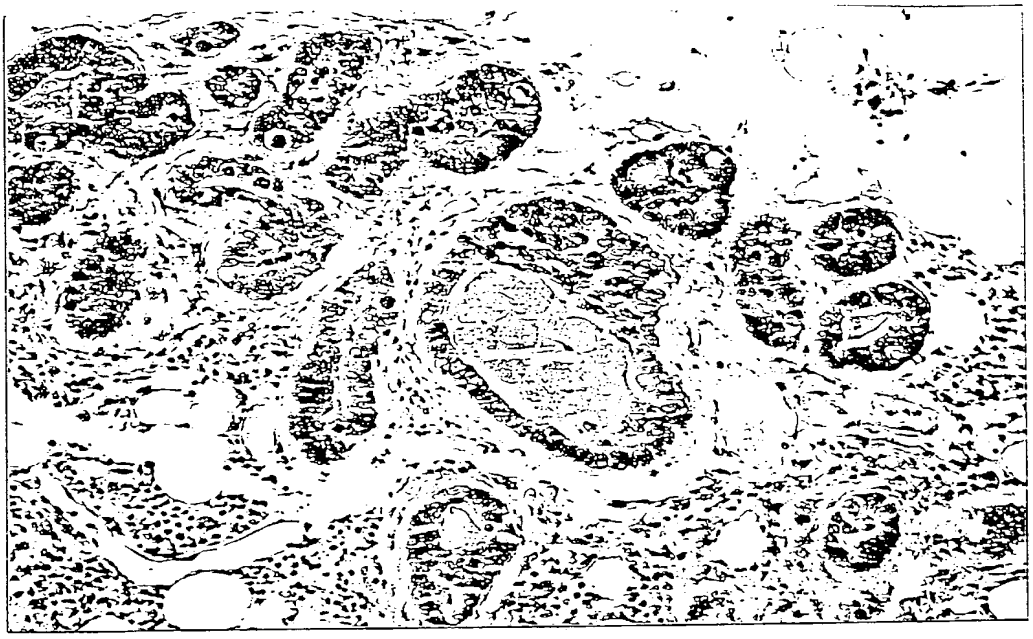

Fig. 6

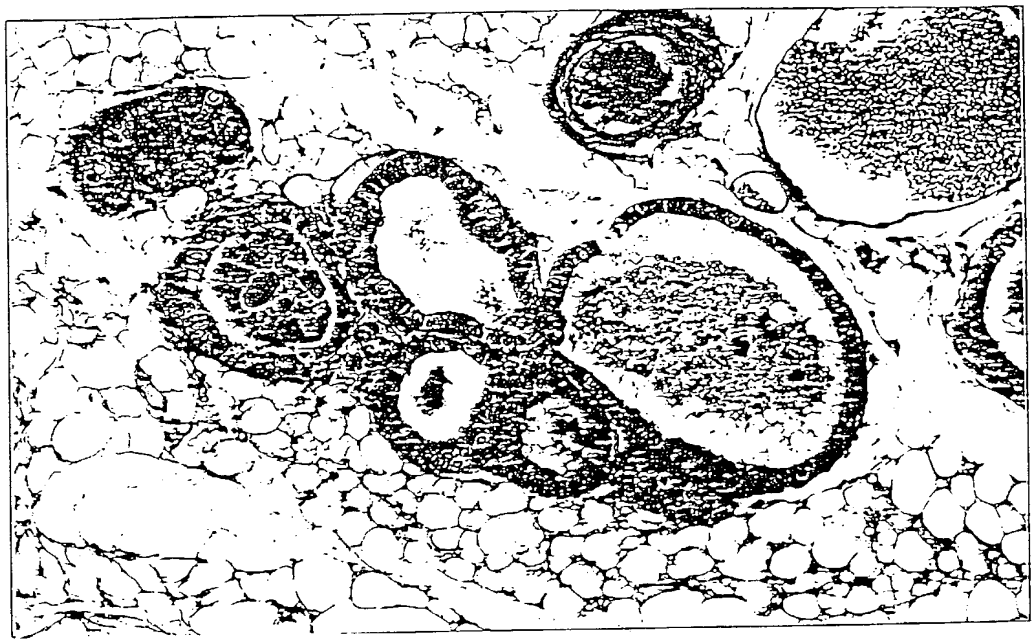

Fig. 7

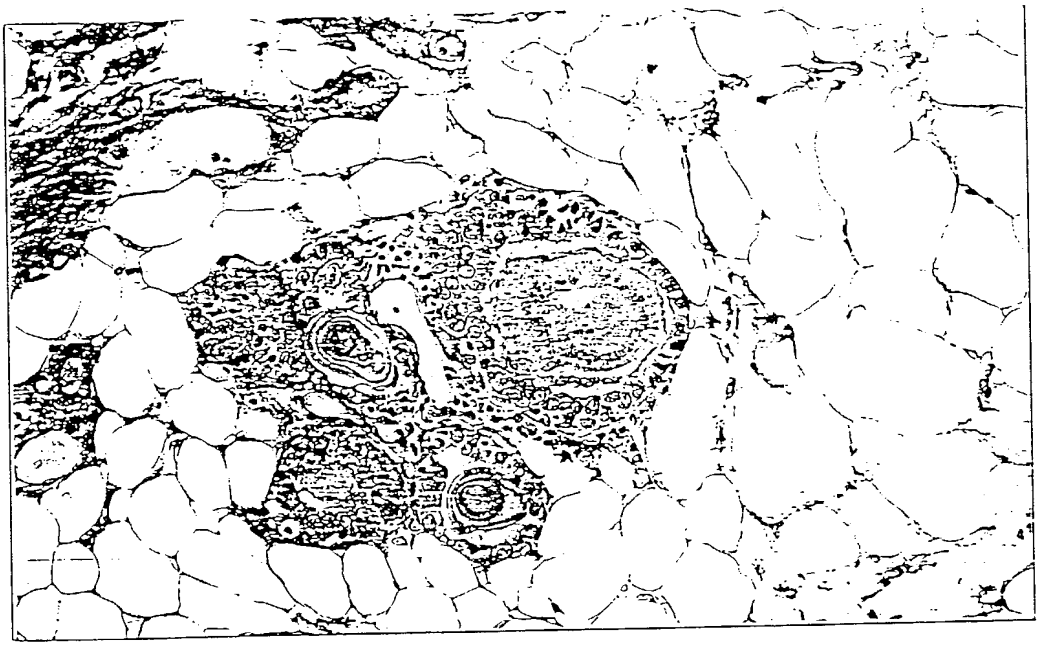

Fig. 8

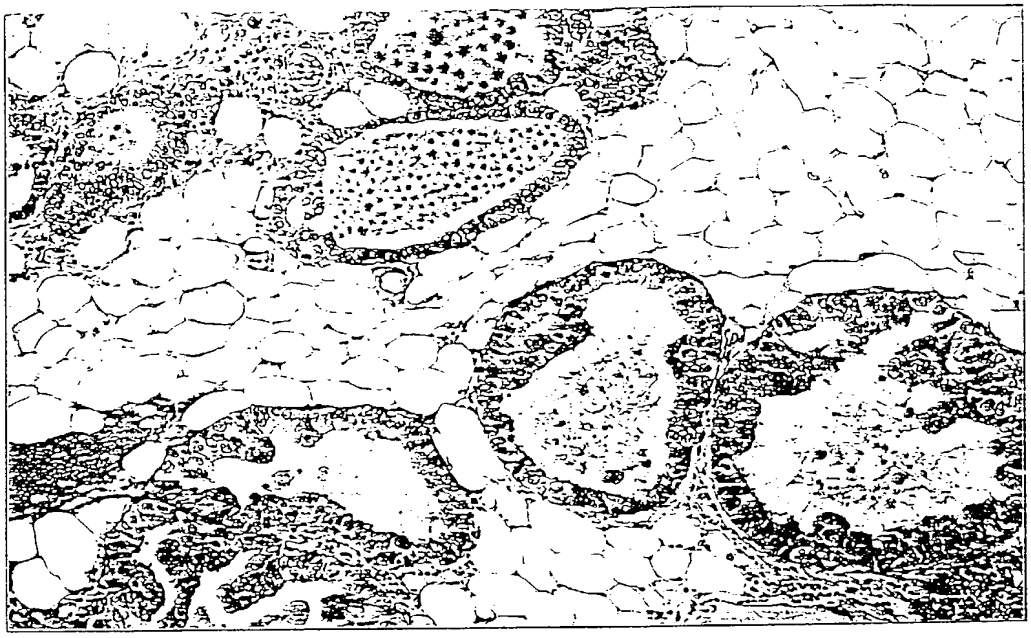

Fig. 9

PATENT APPLICATION

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	ATTORNEY DOCKET NO. 4121-127

As a below named inventor, I hereby declare that:
My residence/post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter, which is claimed and for which a patent is sought on the invention entitled:
MAMMAL, METHOD FOR PRODUCING SAME AND ITS USE
the specification of which is attached hereto unless the following box is checked:
(X) was filed July 23, 2001 as US Application Serial No. 09/889,993 or PCT International Application Number _____ and was amended on _____ (if applicable).
I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose all information which is material to patentability as defined in 37 CFR 1.56.

Foreign Application(s) and/or Claim of Foreign Priority
I hereby claim foreign priority benefits under Title 35, United States Code Section 119(a-d) or 365(b) of any foreign application(s) for patent or inventor(s) certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor(s) certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NUMBER	DATE FILED	PRIORITY CLAIMED UNDER 35 U.S.C. 119
Germany	199 03 371.4	28 January 1999	YES: X NO:
PCT	PCT/DE00/00232	26 January 2000	YES: X NO:

Provisional Application
I hereby claim the benefit under Title 35, United States Code Section 119(e) of any United States provisional application(s) listed below:

U.S. Priority Claim
I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NUMBER	FILING DATE	STATUS(patented/pending/abandoned)

POWER OF ATTORNEY:
As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) listed below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(2) **Steven J. Hultquist, Reg. No. 28021** **Marianne Fuierer, Reg. No. 39983**

Send Correspondence to:	Direct Telephone Calls To:
Steven J. Hultquist Intellectual Property/Technology Law P.O. Box 14329 Research Triangle Park, NC 27709	Steven J. Hultquist (919) 419-9350

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-(2) **Full Name of Inventor:** Christine Schulze-Garg **Citizenship:** German DEX
Residence: ~~Mastagnoweg 7, D-21521 Aumuhle, Germany~~ HARTWITWEG 2, 22529 HAMBURG, GERMANY
Post Office Address: Same

Inventor's Signature **Date** 3rd September 2001

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION (continued) | **ATTORNEY DOCKET NO. 4121-127**

2-00 **Full Name of Inventor:** Wolfgang Deppert **Citizenship:** German DEX

Residence: Im Hain 14, D-22359, Hamburg, Germany

Post Office Address: Same

X W. Deppert — X September 7, 2001

Inventor's Signature **Date**

Full Name of Inventor: _____ **Citizenship:** _____

Residence: _____

Post Office Address: _____

_____ _____

Inventor's Signature **Date**

SEQUENCE LISTING

<110> Schulze-Garg, Christine

<120> MAMMAL, METHOD FOR PRODUCING SAME AND ITS USE

<130> 4121-127

<150> PCT/DE00/00232
<151> 2000-01-26

<160> 2

<170> PatentIn version 3.1

<210> 1
<211> 39
<212> DNA
<213> LCM-Virus

<400> 1
gatcctaggc ctcaagcttc tggagtctac atggatcat
39

<210> 2
<211> 13
<212> PRT
<213> LCM-Virus

<400> 2

Asp Pro Arg Pro Gln Ala Ser Gly Val Tyr Met Asp His
1 5 10